Modeling and Quantitative Analysis of Biological Control Mechanisms

Douglas I. Rouse and Ralph Baker

Former Research Assistant and Professor of Botany and Plant Pathology, respectively, Colorado State University, Fort Collins, CO 80523. Present address of senior author: Department of Plant Pathology, Buckhout Laboratory, The Pennsylvania State University, University Park, PA 16802.

Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree at Colorado State University, Fort Collins.

Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Journal Series Paper 2319.

Contribution to Western Regional Project W-147 which is titled Use of Soil Factors and Soil-Crop Interactions to Suppress Diseases Caused by Soilborne Plant Pathogens.

Accepted for publication 13 March 1978.

ABSTRACT

ROUSE, D. I., and R. BAKER, 1978. Modeling and quantitative analyses of biological control mechanisms. Phytopathology 68:1297-1302.

Cellulose and chitin added to soil induced changes in inoculum potential-disease interactions in Rhizoctonia pre-emergence damping-off of radish. Cellulose added to soil resulted in significant reduction in slope values of the inoculum density-disease incidence (ID-DI) curve when the logarithm of infections was plotted against inoculum density expressed as logarithm of propagules per gram (the log-log transformation). Slope values of the ID-DI curve were near 1.0 for experiments in nonamended soil which is the value predicted if a rhizosphere effect exists for the host-pathogen relationship. Values were not significantly different from 0.67 for experiments in soil to which cellulose had been added; this value is characteristic for a rhizoplane relationship between host and pathogen; i.e., propagules being able to infect only at the surface of the infection court. Thus, a mathematical model involving competition in biological control can be developed to describe the reduction of the rhizosphere to a rhizoplane for damping-off of radish when cellulose is added to soil. In contrast, slope values of ID-DI curves between nonamended and chitin-amended soil did not differ significantly, but the position of the curve was shifted to the right of that for the nonamended soil. This suggests that control is due to the presence of inhibitory compounds following additions of chitin since proportions of propagules participating in infection did not vary with changes in inoculum density. Neither cellulose nor chitin added to soil reduced the population of *R. solani* 9-14 days after application when compared with nontreated controls. Precise differences in efficiency among control measures may be obtained by determining how inoculum potential is influenced by treatments in ID-DI curves.

Mathematical models and computer simulation assist detailed quantitative analyses of epidemiological interactions. There is great interest in this discipline among plant pathologists studying foliar pathogens (15). In contrast, this approach has not been used extensively in studying the disease relationships of soilborne plant pathogens. Nevertheless, methods for epidemiological analysis of soilborne plant diseases are available (2, 3, 4) and data have been gathered in at least one host-pathogen interaction for a more complete systems analysis (7, 8, 9). Models involving preemergence damping-off of radish (*Raphanus sativus* L. 'Early Scarlet Globe') by *Rhizoctonia solani* Kuhn were used to describe the inoculum density-disease incidence (ID-DI) relationship as influenced by environment (8) and survival characteristics of the pathogen (9). The advantage of these models for data analyses is their hypothesized usefulness (2) for describing mechanisms and quantitative analysis of chemical (7), biological (11), or genetic control (12) of plant diseases.

In this paper we report tests on the value of the proposed models in understanding mechanisms and in quantitative analysis of biological control of Rhizoctonia damping-off of radish following soil amendments with cellulose and chitin (7, 8, 9, 13). A short report has been published (18).

00032-949X/78/000 234$03.00/0

MATERIALS AND METHODS

A Fort Collins loamy sand of pH 8.1 (determined colorimetrically in 1:2 soil:0.01 M $CaCl_2$ suspensions) with the following properties was used: organic matter, 1.1%; NO_3^--N, 56.0 μg/g; lime >1%; P_2O_5, 6 μg/g; K_2O 73 μg/g; Fe 17.6 μg/g; and Zn, 3.85 μg/g soil. Part of the adsorption boundary curve of the moisture characteristic of the soil was determined (Fig. 1) using the method of Fawcett and Collis-George (10).

Inoculum of *R. solani* (isolate R3) for addition to soil was grown on a medium (14) of chopped potato (50 g) and soil (500 g) in a 1-liter flask for a minimum of 21 days at 26-30 C before use. Inoculum was removed from the flasks, dried for 24 hr on paper towels, ground with mortar and pestle and screened at 2 mm. The inoculum from one flask was added to 10 kg of raw soil and mixed thoroughly. This infested soil was incubated in a plastic garbage can for 6 days at 24-30 C; 16 kg of raw soil without inoculum was incubated under the same conditions. Chitin (8,000 μg/g soil) or cellulose (25,000

μg/g) were mixed with half of each of these soils (5 kg of infested soil and 8 kg of noninfested soil). The other halves of the soils served as controls. All were incubated in separate cans for an additional 6 days at 26-30 C. The various combinations of these soils were mixed in a twin-shell blender in different proportions, noninfested with infested soil and amended noninfested soil with amended soil infested with *R. solani*. Inoculum densities in the various treatments were determined by a modification of the technique of Ko and Hora (7, 14).

A 200-g layer of soil from the various treatments was placed in plastic trays (13.0 × 13.5 × 3.5 cm) and 50 radish seeds were planted 2 cm apart in each tray using a vacuum planter. Seeds were covered with 0.4 cm of soil, watered to approximately −0.7 bars matric potential, and covered with mylar film. Three applications at each treatment and inoculum level were incubated 6 days at 26-30 C. Moisture varied from 7-13% (−0.1 to −1.0 bar) during the experiments.

Propagules per gram were plotted against percent disease (preemergence damping-off) to determine the range of inoculum densities for each experiment on the logarithmic portion of the inoculum density-disease curve (3, 4). Data were converted to the log-log transformation (6) by plotting the logarithm of propagules/g against the logarithm of infections calculated from the multiple infection transformation for each experiment. Multiple infection corrections were obtained using ln 1/(1-y) in which y represents disease per unit (20). A regression analysis on the logarithmically transformed data was used to obtain straight line fits and slope values.

Populations of *R. solani* over time in nonamended and amended soil were determined by methods previously described (9). Mixtures of soil, inoculum, amendments (chitin, 8,000 μg/g soil or cellulose, 25,000 y/g soil), or appropriate nonamended controls were mixed in a twin-shell blender. Soil samples (400 g/sample) were moistened (−0.7 bars matric potential) and placed in containers fitted with a mylar covering. A needle forced through the cover provided a tiny hole for aeration. Jars were kept in a dark growth chamber at 26-30 C and 65% relative humidity, and subsamples were taken randomly at 2-day intervals to determine the inoculum density of *R. solani* (7, 14). Regression analyses for log-probit and semi-logarithmic transformations of the data were performed as in previous studies (9, 11).

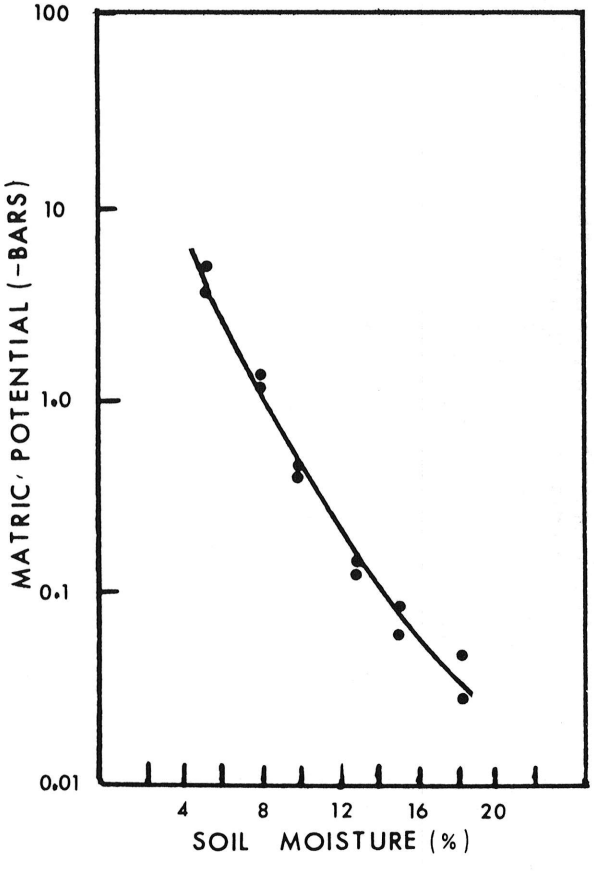

Fig. 1. Matric potential vs. soil moisture curve for soil used in biological control of soil-borne pathogens modeling experiments.

RESULTS

Effect of soil amendments on inoculum density-disease incidence curves.—In repeated experiments slope values of ID-DI curves based on the logarithmically-transformed data were reduced when cellulose was added to soil. Slope values of log-log transformed data for each experiment involving cellulose did not differ significantly from each other ($P = 0.41$). Also, for values of each cellulose treatment, as well as the pooled slope values of all cellulose treatments, were not significantly different from 0.67. Figure 2 represents a composite of the data from three experiments plotted according to the log-log transformation with fitted lines and pooled slopes. The pooled slope for all treatments with nonamended (raw) soil did not differ significantly from 1.0.

Data from a typical experiment comparing ID-DI curves for chitin-amended and nonamended soils is

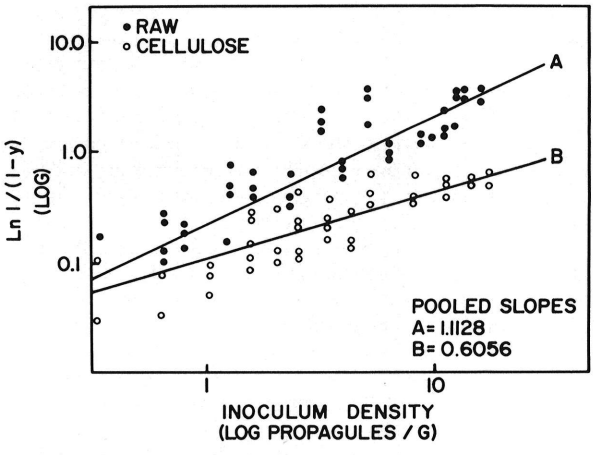

Fig. 2. Pooled log-log slope values of inoculum density-disease curves for preemergence damping-off of radish induced by *Rhizoctonia solani* in nonamended (raw) as compared with cellulose-amended soil. Ordinate scale is in units of infections calculated from multiple infection correction where y is disease incidence per unit (Phytopathology 61:1280-1292).

illustrated for both nontransformed (Fig. 3-A) and transformed (Fig. 3-B) data. Chitin added to soil significantly reduced disease incidence to all inoculum densities (Fig. 3-A). Slope values of log-log transformed curves between amended and nonamended treatments (Fig. 3-B) were not significantly different ($P > 0.25$); however, values were significantly over 1.0 suggesting synergism between propagules as observed frequently in previous experiments (7, 8). The ID_{50} values (inoculum density required for 50% disease incidence) were lower for the raw soil treatment than in soil to which chitin had been added.

There was significant lack-of-fit in all experiments involving additions of cellulose or chitin to soil. An examination of the residuals revealed that lack-of-fit was random and not the result of model error.

Effect of soil amendments on pathogen survival.—Initial inoculum densities of two to three propagules/g soil stayed the same (Fig. 4-A), or fell to a constant level (Fig. 4-B) 3 to 9 days in two experiments (Fig. 4). Semilog transformations of the data produced slope values near zero (Fig. 5-A); however, the log-probit transformation produced a significant regression component (Fig. 5-B). Cellulose amendments had no significant effect on survival when compared with raw soil.

Initial inoculum densities in nonamended and chitin-amended soils for two experiments fluctuated around the initial level for 8-9 days after which the density decreased to a relatively stable value slightly lower than the initial density (Fig. 6). Slope values of data transformed to the semilog (Fig. 6-A) or log-probit (Fig. 6-B) plots were not significantly different between nonamended soil and chitin treatments. In other words, chitin amendments had no significant effect on survival compared with nonamended soil.

DISCUSSION

Diseases induced by *R. solani* conform to a fixed inoculum-fixed infection court configuration (5, 6). In this case, inoculum usually becomes active under the influence of the rhizosphere (1, 8). After correction for multiple infections, the slope value of the ID-DI curve should be near 1.0 in the log-log transformation; however, the slope of the ID-DI curve should be 0.67 if

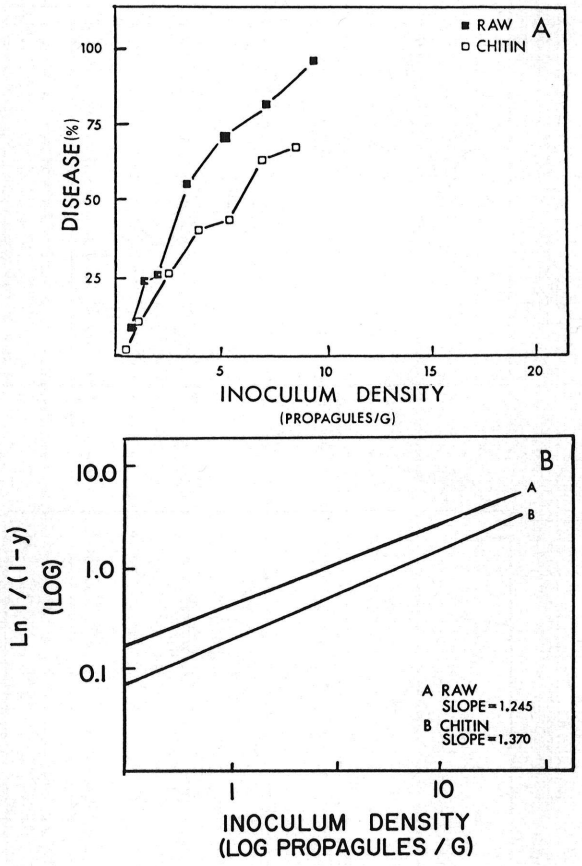

Fig. 3-(A, B). Effect of chitin added to soil on inoculum density disease relationships for preemergence damping-off of radish induced by *Rhizoctonia solani*. The graphs represent **A)** arithmetic plot; **B)** log-log transformation. Ordinate scale is in units of infections calculated from multiple infection correction where y is disease incidence per unit (Phytopathology 61:1280-1292).

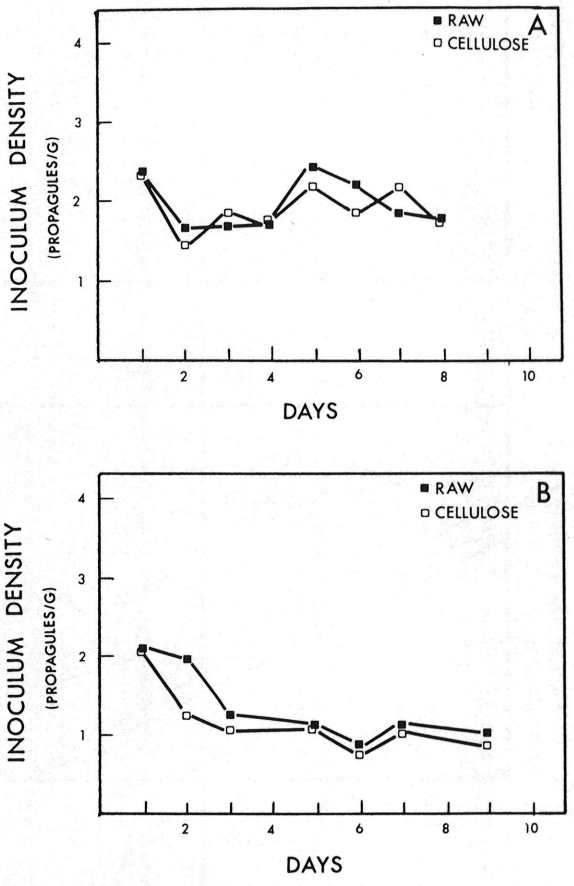

Fig. 4-(A, B). Effect of cellulose added to soil compared with nonamended (raw) soil on survival of *Rhizoctonia solani* in two experiments (**A** and **B**) plotted arithmetically.

inoculum becomes active only on the rhizoplane (6). Slope values of the ID-DI curve reported here for Rhizoctonia preemergence damping-off were near 1.0. When cellulose was added to the soil, the values were reduced and were not significantly different from 0.67.

Biological control induced by adding cellulose to soil has been attributed to the mechanism of competition (1). Soilborne pathogens often require nutrients (usually nitrogen and carbon sources) to germinate, penetrate, and produce successful infection. Usually these become available in the below-ground infection court of the host through exudates from the host or, in the case of nitrogen, also from the soil solution. Cellulose, a pure carbohydrate, soon causes nitrogen, and perhaps other nutrients (1), to be immobilized by proliferating soil microflora thus depriving the pathogen of this important nutrient. Nitrogen-containing compounds released by a germinating seed (or other host infection court) should be available to the pathogen for a relatively short period before becoming immobilized by intense competition for this nutrient in the cellulose-amended soil (16). Thus, nitrogen only becomes available to propagules immediately adjacent to the host infection court–in this case the surface of the seed. In effect then, cellulose added to soil shrinks the nutritional influence of the infection court from a volume extended some distance from the seed to a plane at the surface; in terms of a root, from a rhizosphere to a rhizoplane influence. This explains slope values reduced from near 1.0 to approximately 0.67 in Fig. 2 when cellulose is added to soil.

When chitin was added to soil, the position of the ID-DI curves (log-log transformation) was changed but not the slope (Fig. 3). The mechanism whereby biological control may be induced by addition of chitin to soil is not well understood, but has been attributed to the production of inhibitory or fungistatic substances released during decomposition (19) or to stimulation of a heterolytic microflora capable of digesting the chitinous cell walls of fungi (17). In either case, such mechanisms would not influence rhizosphere-rhizoplane relationships. Relative to this, parallel ID-DI curves (log-log transformation) imply increase of infection rates directly correlated with inoculum density regardless of

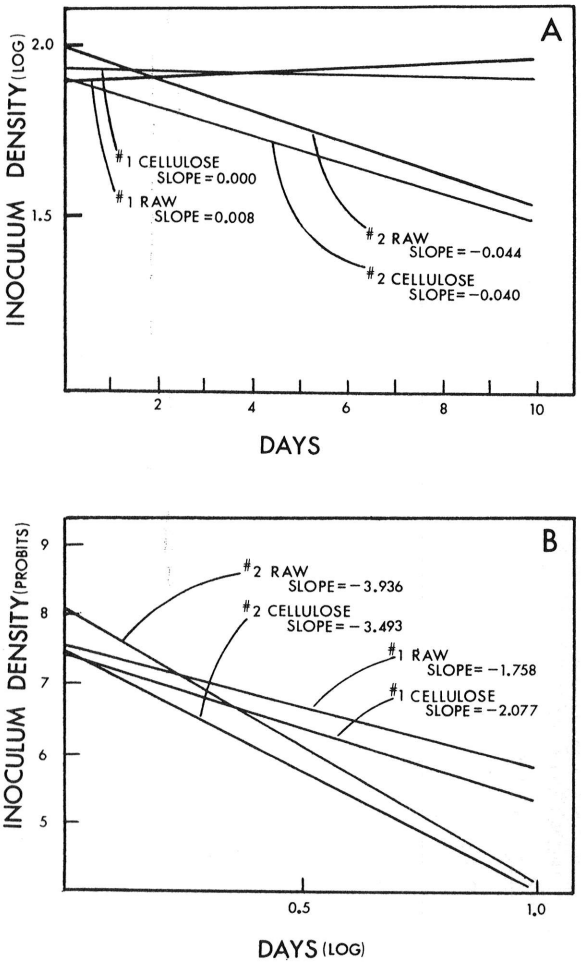

Fig. 5-(A, B). Effect of cellulose added to soil compared with nonamended (raw) soil on survival of *Rhizoctonia solani* in two experiments (#1 and #2): **A)** semilogarithmic transformation; **B)** log-probit transformation.

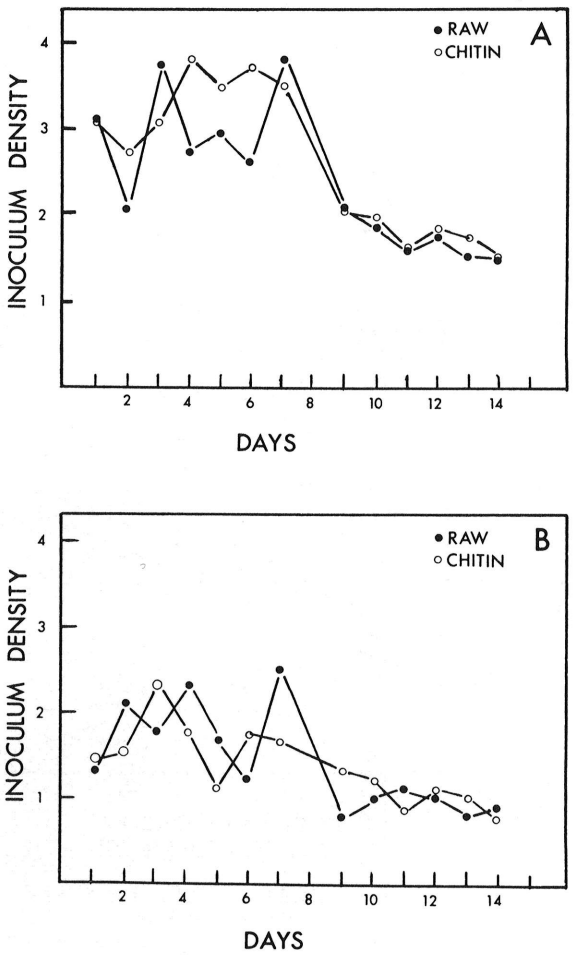

Fig. 6-(A, B). Effect of chitin added to soil compared with nonamended (raw) soil on survival of *Rhizoctonia solani* in two experiments (**A** and **B**) plotted arithmetically.

treatment. In other words, biological (or other) control renders a certain constant proportion of the inoculum (propagules) inactive. Parallel ID-DI curves (log-log transformation) also were observed when biological control of pea wilt was obtained with chitin (11) and when pentachloronitrobenzene was used for chemical control of Rhizoctonia damping-off (7).

Nontransformed and transformed analyses of data indicated no influence of either cellulose or chitin additions to soil on survival of the pathogen (Fig. 4-7). Thus, it was not necessary to incorporate considerations related to survival in modeling the phenomena reported in this paper.

Since ID-DI slope values were different when chitin was used than when cellulose was used, the approach to comparative quantitative analysis of the overall efficiency of the two is not straightforward. The proportion of successful propagules decreased more rapidly with increasing inoculum densities in cellulose-amended than in nonamended soil; a changing rate of successful infections changes the slope so that the distance between curves is not constant. Thus, comparisons are only valid for a given disease and/or inoculum density level (e.g., at the ID_{50} value). Alternatively, it is possible to calculate the efficiency of inoculum in the presence of various treatments. Garrett [quoted in Baker (3)] derived this value from the binomial distribution:

$$p = 1-(1-P)^{1/d} \qquad \text{Eq. 1}$$

where p is the probability that any one propagule will succeed in infection and P is the probability that a host will be infected given a density (d) of propagules. Again this is only valid with comparisons of ID-DI curves of the same slope values: different rates of control with respect to inoculum density provide an insufficient index for amount of control achieved over a range of inoculum densities.

The most comprehensive measure of the amount of control provided by two or more control agents (individually) is to calculate the change of efficiency of inoculum between two or more treatments over all inoculum levels. This is accomplished by calculating the difference between the ID-DI curves generated for amended and nonamended treatments. Thus:

$$\Delta A = \int_{X=I_o}^{X=I} (b_1-b'_1)X+(b_o-b'_o)dx \qquad \text{Eq. 2}$$

where A equals the difference in the area under the ID-DI curve (log-log transformation) because of disease control, b_o and b_1 are regression coefficients for the curve generated in the nontreated control, b'_o and b'_1 are the regression coefficients for the amended treatment, and I_o and I are the inoculum density limits for integration. It is important to standardize the limits of integration so that valid comparisons of data from different experiments are possible. The ID_{10} and ID_{90} values of the raw soil or nontreated control were arbitrarily chosen for this reason.

TABLE 1. Index of the amount of control achieved by each amendment calculated as the change in efficiency of inoculum due to the amendment using Equation 3[a]

Experiment No.	Cellulose	Chitin
1	0.32380	0.28594
2	0.63858	0.40312
3	0.87114	...[b]
Average	0.61117	0.34453

[a]Equation 3 is: $\Delta A = 0.5\,[(b_1-b'_1)\,(I^2-I^2_o) + (b_o-b'_o)\,(I-I_o)$ in which A equals the difference in the area under the ID-DI (inoculum density-disease incidence) curve (log-log transformed data) because of disease control, b_o and b_1 are regression coefficients for the curve generated in the nonamended control, b'_o and b'_1 are the regression coefficients for the amended treatment, and I_o and I are the inoculum density limits of integration. The ID_{10} and ID_{90} values of the nonamended or nontreated control were arbitrarily chosen for the limits of integration.

[b]Comparison of control using chitin was not made.

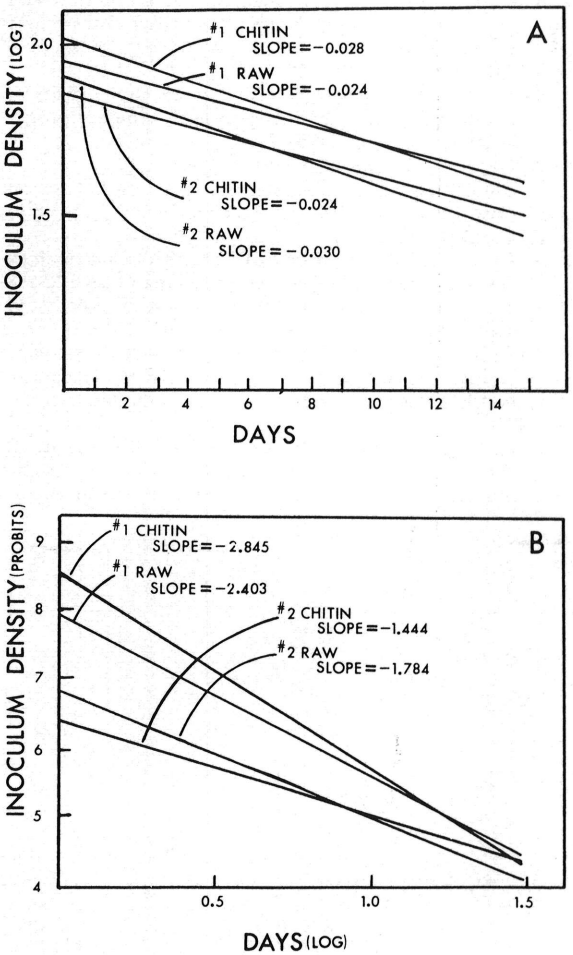

Fig. 7-(A, B). Effect of chitin added to soil compared with nonamended (raw) soil on survival of *Rhizoctonia solani* in two experiments (#1 and #2): **A)** semilogarithmic transformation; **B)** log-probit transformation.

Equation *2* can be written in the following form for computational purposes:

$$\Delta A = 0.5\,[(b_1 - b'_1)\,(I^2 - I^2_o)] + (b_o - b'_o)\,(I - I_o) \qquad \text{Eq. 3}$$

A quantitative analysis of the amounts of control observed using additions of cellulose or chitin to soil is given in Table 1. Overall, the addition of cellulose to soil reduced efficiency of inoculum more than chitin.

Quantitative analyses and modeling as developed in this paper, provide a means for precise relative measurements of the amount of control achieved and allow accurate evaluations useful in comparisons and in integrated control.

LITERATURE CITED

1. BAKER, R. 1968. Mechanisms of biological control of soil-borne pathogens. Annu. Rev. Phytopathol. 6:263-294.
2. BAKER, R. 1970. Use of population studies in research on plant pathogens in soil. Pages 11-15 *in* A. T. Toussoun et al., eds. Root diseases and soil-borne pathogens. Univ. Calif. Press, Berkeley. 252 p.
3. BAKER, R. 1971. Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
4. BAKER, R. 1978. Inoculum potential. Pages 137-157 *in* J. G. Horsfall and E. B. Cowling, eds. Plant pathology: an advanced treatise, Vol. II. Academic Press, New York. p. (In press)
5. BAKER, R., and C. A. MARTINSON. 1970. Epidemiology of diseases caused by Rhizoctonia solani. Pages 172-188 *in* J. R. Parmeter, Jr., ed. Rhizoctonia solani, biology and pathology. Univ. Calif. Press, Berkeley. 255 p.
6. BAKER, R., C. L. MAURER, and R. A. MAURER. 1967. Ecology of plant pathogens in soil. VIII. Mathematical models and inoculum density. Phytopathology 57:662-666.
7. BENSON, D. M., and R. BAKER. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: influence of pentachloronitrobenzene. Phytopathology 64:38-40.
8. BENSON, D. M., and R. BAKER. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: inoculum potential and disease potential interaction. Phytopathology 64:957-962.
9. BENSON, D. M., and R. BAKER. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: survival. Phytopathology 64:1163-1168.
10. FAWCETT, R. G., and N. COLLIS-GEORGE. 1967. A filter paper method for determining the moisture characteristics of soil. Aust. J. Exp. Agric. Anim. Husb. 7:162-167.
11. GUY, S. O., and R. BAKER. 1977. Inoculum potential in relation to biological control of Fusarium wilt of peas. Phytopathology 67:72-78.
12. HANOUNIK, S. B., W. R. PIRIE, and W. W. OSBORNE. 1977. Influence of soil chemical treatment and host genotype on the inoculum-density relationships of cylindrocladium black rot of peanut. Plant Dis. Rep. 61:431-435.
13. HENIS, Y., A. GHAFFAR, and R. BAKER. 1978. Integrated control of Rhizoctonia damping-off of radish: effect of successive plantings. PCNB, and Trichoderma harzianum on pathogen and disease. Phytopathology 68:900-907.
14. KO, W. H., and F. K. HORA. 1971. A selective medium for the quantitative determination of Rhizoctonia solani in soil. Phytopathology 61:707-710.
15. KRAUSE, R. A., and L. B. MASSIE. 1975. Predictive systems: modern approaches to disease control. Annu. Rev. Phytopathol. 13:31-47.
16. MAURER, C. L., and R. BAKER. 1965. Ecology of plant pathogens in soil. II. Influence of glucose, cellulose, and inorganic nitrogen amendments on development of bean root rot. Phytopathology 55:59-72.
17. MITCHELL, R., and M. ALEXANDER. 1963. Lysis of soil fungi by bacteria. Can. J. Microbiol. 9:169-177.
18. ROUSE, D., and R. BAKER. 1976. Verification of a model for biological control of Rhizoctonia solani pre-emergence damping-off. Proc. Am. Phytopathol. Soc. 3:220 (Abstr.).
19. SNEH, B., J. KATAN, and Y. HENIS. 1971. Mode of inhibition of Rhizoctonia solani in chitin-amended soil. Phytopathology 61:113-117.
20. VAN DER PLANK, J. E. 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 p.